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EXPERIMENTS WITH FEEDING THYMUS GLANDS TO FROG LARVÆ.

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INTRODUCTION.

In 1912, J. F. Gudernatsch published an account of his investigation concerning the effects of feeding various glands of internal secretion to frog larvæ. Among other interesting results observed, was the significant one, that fresh thymus gland, when fed to tadpoles, stimulates the growth processes of these animals and at the same time inhibits the onset of metamorphosis.

In a later communication, January, 1914, this author repeated his previous experiments with thymus feeding, and confirmed his earlier results. For an understanding of the relation between growth and the secretory products of the thymus gland, the importance of the results obtained by this investigator warranted a repetition of his work, which, so far as the writer is aware, has never been questioned. It was with this end in view that the following experiment was undertaken.

LITERATURE.

The experimental researches upon the thymus gland may roughly be divided into two groups: those dealing with the relation between the thymus and bodily growth, and those concerned with the correlation in function of the thymus and sex glands. The literature dealing with the former problem is more extensive than that concerned with the latter.

EFFECTS OF EXTIRPATION UPON GROWTH.

One of the first investigators of this problem was Carbone; this experimenter extirpated the thymus of rabbits, and one dog, and found that the animals did not differ in their development from control animals of the same litter.

Another investigator, Ghika, removed the thymus from eight

cats and thirteen rabbits; he found that in the first few days there was emaciation and arrest of development which lasted for weeks, although, in some of the animals, the losses were afterwards made good.

Cozzolino removed the thymus from young rabbits weighing 300-400 gm., and in the first two months after the operation was unable to detect any change in their condition.

In a later communication, however, he described crookedness of the legs, arrest of growth, and progressive cachexia in one animal upon which he had operated three months previously.

S. Vicent extirpated the thymus of guinea pigs and did not observe any changes in their condition.

Paton and Goodall found that extirpation of the thymus produced no effect upon the general growth and development of new born guinea pigs.

R. Fischel extirpated the thymus of goats, rabbits and dogs, and obtained negative results. He concluded from his experiment, that thymectomy has no effect upon the development of the subject.

The work of this author has been severely criticized by Basch, who claims that the experimental conditions were inappropriate.

Basch extirpated the thymus of suckling dogs and obtained, striking changes in the growth of his thymectomized animals. The most important growth changes were those affecting the skeleton.

U. Soli found that thymectomy was followed in young rabbits by changes in the development of the skeleton, these changes affecting the hollow, long bones and ribs. This author failed to confirm the results obtained by Basch, with guinea pigs, in which thymectomy is a comparatively simple operation. This would seem to indicate that the symptom complex, observed by Basch following thymectomy in his animals, was probably due to post-operative changes and not connected with the thymus, because in those animals used by Basch, thymectomy is a very severe operation.

Halnan and Marshall extirpated the thymus of young guinea pigs, and found that removal had no influence upon the growth of these animals.

Pappenheimer extirpated the thymus of young albino rats and found that removal had no effect upon body growth and development.

Adler extirpated the thymus of tadpoles and found that these animals suffered no ill effects from the operation. Growth and development was unaffected.

(The work of Gudernatsch has been noted.)

This rather extensive review of the literature was thought justifiable in view of the discrepancy in the results obtained by the various investigators.

MATERIAL AND OBSERVATIONS.

The following experiment began May 3, 1915, and was completed July 29, 1916. The work in 1915 dealt with the effect of feeding thymus powder to tadpoles, the work for 1916 was concerned with those experiments in which only the fresh gland was employed as food. I shall discuss the work dealing with the fresh gland first, and devote the latter part of this paper to feeding experiments with thymus powder.

FEEDING FRESH THYMUS GLAND.

This work began April 12, 1916. April 2, the eggs of *Rana pipiens* were gathered from a group of shallow pools near the University campus at Lawrence, Kansas, and allowed to develop in the laboratory. One group of eggs was hastened considerably in development by keeping them in a warm room. When the young larvae had emerged from their gelatinous egg capsules and developed to the free feeding stage, they were placed in glass bowls ten inches in circumference by four inches deep. The tadpoles were covered with ordinary tap water which was changed daily.

When the larvae had attained a length of nearly 13 mm., one hundred and fifty were selected, and divided into two groups of seventy-five each; one lot was destined for thymus feeding, the other for control purposes. These two lots of seventy-five animals were then subdivided into lots of ten and placed in glass bowls, one lot to a bowl. Both control and experimental larvae were reared under identical conditions as regards temperature, light and water supply.

A second series of larvæ, one hundred in number and averaging 10 mm. in length, were selected and treated the same as the series above. Fifty larvæ were for thymus feeding and fifty for control purposes. These larvæ were from a batch of eggs gathered a few days later than the larvæ of the first series. Hence, throughout the following experiment, the series averaging nearly 13 mm. in length when started upon the thymus feeding I shall designate as Thymus No. 1, the series averaging 10 mm., at the beginning of the work, as Thymus No. 2.

TABLE I.

APRIL 12.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
12	11	10.5	10
12.5	13	11	9
11.5	12	9	11
13	11	10	9.5
13	12	10.5	10.5
13.5	13	10	10
12.5	13.5	9	9
11	13	11	9.5
12	12.5	9.5	9
12.5	11.5	10.5	10.5
11	14	10	11
11	13	11	11.5
13	13	10	10.5
13.5	14	10.5	11.5
12.5	13.5	9.5	11
11.5	11.5	9	11.5
11	12.5	11	10
12	13	9.5	10.5
13	13.5	10	9.5
13	13	10.5	10
Av. len.....12.25	12.6+	10.1	10.25

The experimental larvæ of both series were each day fed finely shredded bits of the fresh thymus glands of calves 3-12 months of age. Only the glands of young animals were used. The glands were obtained from a nearby slaughter-house, and extirpated by myself.

The control animals were fed fresh beef liver each day. Care was taken to give as nearly as possible the same amount of protein material to the animals in each container and of each

group. Both thymus- and beef-fed larvæ ate greedily the food given them.

At the beginning of the experiment twenty larvæ of both the control and thymus-fed groups of larvæ were measured with a millimeter rule, and the average length computed. This seemed a satisfactory method of procedure, considering that all of the larvæ came from the same batch of eggs, and consequently were of the same age.

A very convenient method of measuring the young larvæ is to lay them upon moistened blotting paper. The moisture in the paper prevents injury to the tail and at the same time the larvæ are unable to lash about.

Table I represents the measurements of twenty larvæ of both control and thymus-fed groups April 12, the day the experiment began.

None of the larvæ measured revealed any indications of limb development when examined under the microscope.

OBSERVATIONS OF APRIL 20.

When examined upon this date no observable differences in color or activity of the control and thymus-fed larvæ were noticed. None of the larvæ possessed limb buds. Considerable growth, however, had occurred in the larvæ of both groups, but in regard to length and body size, both control and thymus-fed animals were about equal, as Table II indicates.

OBSERVATIONS OF APRIL 29.

A careful examination upon this date, of the larvæ of both thymus-fed and control groups failed to reveal any differences in pigmentation, activity, or mortality among the animals. Upon examination under the microscope, tiny limb buds of the posterior extremities were observed on those larvæ of the control group measuring 25 mm. or over. Limb buds were observed on seven of the thymus-fed larvæ measuring 27 mm. and over, in the thymus No. 1 group.

The limb buds of the larvæ at this early stage appeared as two round, blunt projections somewhat anterior and lateral to the anal opening.

TABLE II.

APRIL 20.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
18	18	14	15
19	18.5	15	15.5
18.5	19	14.5	15.5
18.5	19	14	14.5
18	19.5	15.5	16
20.5	19.5	16	14
19.5	18.5	16	14.5
20	17.5	15	15
18.5	18.5	15.5	14.5
18	17	14.5	16
19	18	16	16.5
19.5	19.5	15	15
18	19.5	15	15.5
18	18.5	14.5	16
17.5	18	14.5	15
18.5	20	14	15.5
17.5	17.5	16	15.5
19	18	15.5	16
19.5	18.5	15	14.5
18	20	15.5	15.5
Av. len.....18.65		18.62+	15.05
			15.22+

The length of the larvæ in both control and thymus-fed animals was about the same though several slight discrepancies are revealed in Table III. The thymus No. 1 group shows a slight acceleration in growth (average length) when compared with their liver-fed controls. This acceleration was not shown by the thymus No. 2 group. These small growth differences are shown in Table III.

The slight difference in the length of the thymus-fed and control animals may possibly be due to the fact that thymus tissue is somewhat more compact than liver tissue and probably presented greater difficulty for the tadpole in eating. Both kinds of tissue were, however, greedily devoured by the larvæ.

OBSERVATIONS OF MAY 4.

Very faint differences in pigmentation were noticed between the larvæ of the thymus-fed and control groups. The thymus-fed animals were slightly darker than their controls which had a reddish tinge. As regards activity, body-shape and mortality,

TABLE III.

APRIL 29.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
26 l.b.	25.5 l.b.	23	22
25 "	26 "	22.5	23.5
24.5	25 "	21.5	23
28 "	25.5 "	22.5	22.5
28 "	25 "	22	22
25	25.5 "	23.5	21.5
29 "	30 "	22	21.5
25	25.5 "	21	22.5
26.5	24.5 "	21.5	22.5
27 "	29 "	23	22
27 "	23.5	23.5	23.5
25	24	22.5	23
25	23	22	23.5
24.5	24.5	22	22.5
24.5	23.5	23.5	22
26 "	25 "	23	23.5
25	23.5	22	22
27	24	23.5	22
26	24	22.5	23.5
25.5	23	22	22.5
Av. len... 25.97	24.97 ¹	22.45	22.55

no differences between the animals of the two groups were observed. When examined under the microscope, both the control and thymus-fed larvæ had hind limb buds. The limbs had not as yet differentiated into their two primary divisions, nor had the toe points developed. The limb buds of both groups of animals appeared to be of nearly the same size and in the same stage of development. The thymus No. 2 group of larvæ had by this time outstripped the thymus No. 1 group in growth.

The table below shows that the control animals of the thymus No. 1 group averaged, upon this date, somewhat greater length than any of the thymus-fed larvæ. The animals of the thymus No. 2 group were somewhat larger than their controls.

OBSERVATIONS OF MAY 8.

The thymus-fed larvæ upon this date were distinctly darker in color than their controls; no other differences were observed in the two groups of tadpoles. Both controls and thymus-fed

¹ l.b. = limb buds.

TABLE IV.

MAY 4.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
28 l.b.	29 l.b.	31 l.b.	26 l.b.
30 "	31 "	33 "	33.5 "
29 "	37.5 "	34.5 "	34 "
28.5 "	34.5 "	32.5 "	25.5 "
27.5 "	33 "	33 "	32.5 "
32 "	30.5 "	27.5 "	32 "
34 "	28 "	30.5 "	30 "
28.5 "	37 "	30 "	26.5 "
34.5 "	30 "	35 "	33 "
32 "	33.5 "	32 "	29 "
28.5 "	30.5 "	33.5 "	25.5 "
29.5 "	37 "	37 "	33 "
34 "	33.5 "	31.5 "	30 "
30 "	34.5 "	30.5 "	29 "
26.5 "	32.5 "	30 "	25.5 "
33 "	34.5 "	31.5 "	29 "
31 "	34 "	32 "	27.5 "
27.5 "	32 "	32.5 "	30 "
30 "	30.5 "	34 "	31 "
34 "	33 "	33 "	32 "
Av. len....30.42	32.8	32.22	29.72

larvæ possessed hind limb buds; these were plainly visible without the aid of a hand lens. The larvæ of both groups of animals had increased in size considerably but the average length of the larvæ of each group varied but little, and such variations are negligible. This is clearly indicated in Table V.

OBSERVATIONS OF MAY 13.

When examined the larvæ of both control and thymus-fed groups had increased in length considerably. All of the tadpoles of both groups had developed hind limb buds and most of the limb buds of the thymus-fed larvæ had differentiated into their two primary divisions and had developed the toe points. The controls seemed somewhat behind the thymus-fed larvæ in respect to the length of the limbs. The pigmentation of the experimental animals was darker than the liver-fed controls.

In other respects the larvæ of the two groups appeared the same. Table VI indicates that the larvæ of all groups averaged nearly the same length. The differences are negligible.

TABLE V.

MAY 8.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
31.5 l.b.	31.5 l.b.	31.5 l.b.	35 l.b.
30.5 "	37 "	33 "	31.5 "
31 "	34 "	34 "	32 "
30.5 "	37 "	32.5 "	33.5 "
30 "	34 "	34 "	30.5 "
36.5 "	33 "	35 "	30 "
40.5 "	37.5 "	34 "	35.5 "
29 "	38 "	33 "	34 "
30 "	33 "	33.5 "	32.5 "
33.5 "	30.5 "	32 "	30 "
33 "	37 "	33 "	32 "
32 "	33 "	37 "	33 "
30.5 "	34.5 "	34 "	31.5 "
33 "	32 "	33 "	30.5 "
29.5 "	33 "	30.5 "	29.5 "
29 "	31.5 "	35.5 "	31.5 "
30.5 "	33 "	32 "	32 "
33.5 "	34 "	31.5 "	32.5 "
32.5 "	32 "	33 "	33 "
34 "	31.5 "	30.5 "	33 "
Av. len....32.02		33.85	33.12
			32.15

TABLE VI.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
37.5 l.b. p.d. ¹	37 l.b.	40 l.b.	44 l.b. p.d.
44 " p.d.	36.5 "	45 " p.d.	45 " p.d.
37 "	42 " p.d.	37 "	39.5 "
33.5 "	33.5 "	37.5 " p.d.	40 "
36.5 "	40 " p.d.	35 "	33.5 "
33 "	35.5 "	33.5 "	45 " p.d.
35.5 "	35 "	33.5 "	40 " p.d.
32 "	37 "	32 "	35.5 "
35 "	38 " p.d.	35.5 "	43 " p.d.
32.5 "	37 "	40 " p.d.	38 "
35.5 "	40 " p.d.	40 "	35.5 "
42 " p.d.	48 "	41.5 " p.d.	39 "
40 "	35.5 "	37 "	38 "
42 " p.d.	33.5 "	38 "	42 " p.d.
43 " p.d.	35 "	34.5 "	42.5 " p.d.
36.5 "	34 "	37.5 " p.d.	41 " p.d.
37 " p.d.	35.5 "	35 "	35.5 "
35.5 "	37 "	34.5 "	34.5 "
34 "	38 " p.d.	37 " p.d.	39 "
39 " p.d.	36.5 "	35 "	37 "
Av. len. 37.05		37.22 +	36.95
			39.37 +

¹ The letters p.d. indicate that the limb buds have differentiated into their two primary divisions.

OBSERVATIONS OF MAY 20.

Examination of the larvæ at this date revealed no difference between the animals of the control and thymus-fed groups as regards pigmentation, body shape, size or activity. The limb buds of both groups had by this time differentiated into their two primary divisions, and had well developed toe points. The size of the limbs of both groups appeared to be the same. The dark pigmentation of the thymus-fed larvæ had gradually faded out, and was no longer visible on May 20. Table VII shows the average lengths of the animals of the experimental and control groups. The average length is nearly the same in all groups. The greatest difference in length between the thymus-fed and control larvæ was about two millimeters.

TABLE VII.

MAY 20.

Length.			
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.	Thymus No. 2, Mm.	Control of Thymus No. 2, Mm.
46 l.b. p.d.	45 l.b. p.d.	44 l.b. p.d.	41 l.b. p.d.
45 " "	45 " "	47 " "	42.5 " "
38 " "	47 " "	39 " "	44 " "
46 " "	45 " "	43 " "	39 " "
37.5 " "	35.5 " "	41.5 " "	38.5 " "
39 " "	37 " "	40.5 " "	41.5 " "
48.5 " "	34.5 " "	45.5 " "	39 " "
37 " "	44 " "	42 " "	40.5 " "
41 " "	46 " "	43 " "	40.5 " "
39 " "	37.5 " "	47 " "	41 " "
39 " "	44 " "	46.5 " "	39 " "
42 " "	37.5 " "	44.5 " "	41.5 " "
40 " "	46 " "	38.5 " "	43 " "
44 " "	35.5 " "	40.5 " "	44 " "
37.5 " "	46 " "	39 " "	40.5 " "
39 " "	44 " "	38 " "	41 " "
38 " "	43 " "	44 " "	39.5 " "
39.5 " "	37.5 " "	42 " "	41.5 " "
40.5 " "	38 " "	41 " "	38.5 " "
41 " "	39.5 " "	39 " "	40 " "
Av. len. 40.8	41.37	42.27	40.8

All larvæ which had developed the two primary divisions of the legs in this series, had well-developed toe points.

The larvæ were not measured again until June 8, eighteen days from the date of previous measurement. The larvæ had

grown so large that it was considered best to avoid handling them, as much as possible. During the eighteen-day interval only four tadpoles died. One was a control and the other three were thymus-fed animals. The four larvæ became bubbly, *i. e.*, air vacuoles appeared in the region of the lungs under the skin. The larvæ came to the surface and floated on their backs; death usually resulted within a few days. The following table indicates the measurements of the larvæ.

TABLE VIII.

OBSERVATIONS JUNE 8.

Measurements of the Larvæ.							
Thymus No. 1, Mm.	Control of Thymus No. 1, Mm.		Thymus No. 2, Mm.		Control of Thymus No. 2, Mm.		
50 Legs 3.5	54.5	Legs 6.5	47.5	Legs 3	50	Legs 2.5	
47 " 3	56	" 10	57	" 4	50	" 2.5	
57 " 8	50.5	" 9	55	" 4	50.5	" 2	
49 " 2	60	" 10	48	" 3	55.5	" 3	
60 " 8	45.5	" 2	44.5	" 2	55	" 4.5	
52 " 3	43.5	" 8.5	60	" 10	54.5	" 6.5	
48 " 2.5	58	" 8	55	" 4.5	51.5	" 2	
55 " 2	58	" 10.5	42.5	" 2	53	" 3.5	
45 " 2.5	57	" 6	50	" 3	47.5	" 2	
58 " 2	60	" 7	53	" 3.5	55	" 4	
49 " 2.5	56	" 5.5	41	" 2	45.5	" 3	
60 " 3	48	" 3	58	" 7.5	53.5	" 5.5	
51 " 4	52	" 4	50.5	" 5	53	" 5	
53 " 3	54	" 4	58	" 5	58.5	" 9	
58 " 6.5	45	" 2.5	50	" 2	56	" 6.5	
57 " 9	59	" 3.5	55	" 3.5	52	" 4	
55 " 6	48	" 2	55	" 4	45.5	" 2.5	
50 " 5	49.5	" 2.5	60	" 8	49	" 2.5	
56 " 5.5	51.5	" 3	50	" 2	51.5	" 3	
60 " 6	53	" 4	62	" 11	53	" 5.5	
Av. len. 53.95	4.35	52.95	5.57+	52.6	4.45	52.	3.95

OBSERVATIONS OF JUNE 8.

The larvæ of both control and thymus-fed cultures had grown considerably in the eighteen-day interval between the observations of May 20 and June 8. All of the tadpoles of both groups possessed well-developed limbs, some measuring 11 mm. in length. The limbs of the thymus-fed animals averaged in length about the same as the controls; this was true also of body length, as a glance at Table VIII. will show.

In regard to pigmentation, activity and mortality among the

animals of the two groups, practically no differences were observed. The forelimbs of the larvæ had not, as yet, broken through the skin, but showed as slight bulges or swellings under the skin of the pectoral region. The growth of all the larvæ had been retarded, no doubt, by unseasonably chilly weather.

June 10, all of the larvæ of both thymus and liver-fed cultures, on account of their increased size, were removed from their containers and placed in very much larger basins. The new containers were especially constructed sinks, measuring $30 \times 18 \times 6$ inches. Thirty larvæ were placed in each sink, which were so arranged that fresh, well-oxygenated water flowed through them constantly.

By June 20, two of the control larvæ, and one of the thymus-fed group, were in advanced stages of metamorphosis. The forelimbs appeared and the tail was reabsorbed. June 26, these larvæ had completely metamorphosed. From this date on, metamorphic changes appeared in almost all of the larvæ, both of the control and thymus-fed groups. By July 3, six thymus-fed and eight liver-fed larvæ had metamorphosed, and by July 12, most of the larvæ had already metamorphosed, or else were in advanced stages of the process.

The animals were all killed, and their gonads preserved for microscopic examination. Macroscopic examination of the germ glands of both thymus-fed and control animals revealed no observable differences either as regards the size of the gland or proportion of the sexes.

The gonads were fixed in Flemming's fluid and sectioned at a thickness of 7.5μ . The sections were stained with iron alum haematoxylin, counter stained with congo red. Only four of the gonads were examined microscopically, two from thymus-fed and two from control larvæ.

Microscopic examination of the four gonads revealed nothing of interest. Three of the gonads were from female larvæ, and contained young oöcytes undergoing growth. The single male gland contained only spermatogonia and was of normal size. The thymus feeding appeared to have had no effect upon the growth and development of the gonads and the germ cells.

We may say, then, in the light of this experiment, that feeding

fresh thymus gland to the larvæ of *Rana pipiens* has no effect upon the growth and metamorphosis of these animals, and the gonads appear to be unaffected by such feeding.

PART II. EXPERIMENTS WITH FEEDING THYMUS POWDER.

The following experiment was started May 3, 1915: The larvæ of *Rana pipiens* were used for the first half of the work. These larvæ had been reared from the egg in the laboratory and were all from one batch of eggs. The feeding work was begun when the tadpoles averaged 28 mm. in length. Most of the larvæ had tiny limb buds of the posterior extremities, at this time, though, as yet, the buds had not differentiated into their two primary divisions. Two series of animals were used, one for feeding work, the other for controls. Both groups were kept under uniform conditions of temperature, light, and water supply. Finger bowls were used as containers, five larvæ to a bowl. Tap water was used to cover them and was changed daily.

The animals of the control group were, each day, fed fresh water algæ, and dried flour paste. This same food was also given to the thymus-fed larvæ, though in smaller amounts in order to obviate any possibility of inanition.

The thymus used was the desiccated glands of sheep prepared by Armour & Co. This powder was mixed with wheat flour in the proportions of three grams of thymus to five grams of flour, enough water was added to make a thick paste which was allowed to dry at room temperature. Pieces of the dried thymus paste about the size of one's thumb nail were finely crumbled and fed to the larvæ. The mixture seemed palatable to the tadpoles for they ate it greedily. The same amount of paste was given each day.

The control animals grew rapidly, and in a few days the limb buds had differentiated into small legs. The development of the thymus-fed larvæ was somewhat slower and, in regard to length, were two or three millimeters behind their controls. On the whole, however, growth in the two series of larvæ was approximately equal, when the fact is considered that the thymus-fed larvæ received less algæ than the controls.

The limbs of the thymus-fed tadpoles differentiated somewhat

more slowly than the limbs of the controls, and appeared to be smaller in size. There were no observable differences in the pigmentation of the two groups.

This series of larvæ was kept until June 3, just one month from the date the thymus feeding began. They were measured on this date, the control larvæ averaging 39.5 mm., the thymus-fed larvæ 37.5. The tadpoles were then killed and preserved for microscopic examination. It is evident that the thymus feeding did not accelerate growth in the animals of this group. The two millimeters difference between the average length of the control group and the average length of the thymus-fed group is, in animals so variable in their growth capacities as frog larvæ, negligible.

On May 9, a second series of frog larvæ were started upon the thymus diet, appropriately controlled. The controls and thymus-fed larvæ of this series averaged 35 mm. All of the larvæ had hind legs though they were very small, and in most of them the toe points were just appearing. These animals were fed the same kind, and amount of food, as the first series received.

The thymus feeding continued for eighteen days, during this period both controls and thymus-fed larvæ grew rapidly, but as recorded for the first series, the controls differentiated limbs faster.

The length of the two groups of tadpoles was approximately equal, both groups averaging 41.5 mm. eighteen days after the experiment began.

Two other series of larvæ were started later, but the tadpoles were too far along in metamorphosis. No results worth recording were obtained.

August, 1915, I obtained some larvæ of the common bull-frog, *Rana catesbeiana*. When found, these tadpoles averaged 32 mm. in length. All except six were used for another experiment. The six remaining larvæ were divided into lots of three each, one for controls, the other for thymus feeding. The nature of the food, and amount fed was the same as that recorded for earlier experiments.

For twenty-one days, this series of tadpoles was fed thymus, and at the end of that period, there was no appreciable difference

between the controls and thymus-fed animals either in regard to body length, shape or stage of development. Before the experiment was discontinued the larvæ of both groups of animals were measured. The controls measured in length 41 mm., the thymus-fed animals 39.5 mm.

In this series of larvæ, as in the two previous series, thymus feeding certainly did not accelerate growth.

On November 1, another set of *Rana catesbeiana* larvæ were obtained, and all larvæ of 75 mm. in length were selected for thymus feeding. There were twelve 75 mm. larvæ. They were divided into two groups of six each for controls and thymus feeding purposes. The diet of both groups was similar to that given to the larvæ of the previous series.

November 10 the animals of both control and thymus-fed groups were measured. The controls averaged 83 mm. in length; the thymus-fed larvæ 85 mm. The limb buds of both groups were very small. No other differences in the two groups were noticed.

December 9, the larvæ of both groups were again measured. The controls averaged 89.5 mm. The thymus-fed larvæ averaged 90.5 mm. Pigmentation, body shape, were the same in the animals of both groups.

December 23, the animals of both control and thymus-fed groups were again measured. The controls averaged in length 92.5 mm., the thymus-fed larvæ 93 mm. The limb buds of both groups appeared to be of about the same size and stage of development.

The experiment was discontinued at this time.

It was observed in this series that the thymus-fed larvæ averaged slightly more in length than the controls. The differences, however, are very small.

A last series of feeding experiments with powdered thymus was started November 17, 1915. Eighty small tadpoles (species not determined), averaging 17 mm. in length and without limb buds, were brought to the laboratory on this date. Thirty were selected and treated similarly to those of the earlier experiments. These animals were fed thymus from November 17 until December 7. Upon this date the tadpoles of both control and thymus-

fed groups were measured. The controls averaged 29.5 mm., the thymus-fed larvæ 30 mm. The disparity in the average lengths of the two series is negligible. The fact to be noticed is that both experimental and control larvæ grew considerably and that both groups possessed tiny limb buds on December 7, when the experiment was discontinued.

SUMMARY OF THYMUS POWDER EXPERIMENT.

To sum up the results obtained in the experiments just recorded it may be said that feeding powdered thymus glands to amphibian larvæ of the species *Rana pipiens*, *Rana catesbeiana* and *Rana* _____ (species undetermined) does not accelerate growth beyond the normal rate. Some of the observations recorded do, however, indicate that thymus feeding may have a slight inhibitory effect upon limb development. However, in animals so extremely variable in their growth capacities as frog larvæ, to attribute such a slight retardation in limb development to the effects of thymus feeding would be rather unsafe. Aside from this extremely slight retardation in limb development of the thymus-fed larvæ, no results were obtained which would indicate that the feeding of powdered thymus gland to amphibian larvæ has any effect upon growth.

SUMMARY AND CONCLUSION.

To sum up the results of this experiment, feeding thymus gland, either in the fresh state or in the powdered form, to frog larvæ of the species *Rana pipiens*, *catesbeiana*, and one undetermined species, does not accelerate the growth processes, or retard the metamorphosis of these animals. The tadpoles seem to develop normally in every respect. The gonads do not appear to be effected by thymus feeding.

These results are at variance with the findings of Gudernatsch. It may be, however, that the species of frog used by this investigator reacts differently to thymus feeding than does *Rana pipiens* and *catesbeiana*, thus accounting for the disparity of the results obtained by this investigator and myself.

One point, however, must not be overlooked in this connection, *i. e.*, that the variability of the growth rate in frog larvæ is

very great, even in larvæ reared from the same batch of eggs and under similar conditions. Very slight environmental changes are sufficient to bring about fluctuations of the processes of growth in these animals, and even when the changes are reduced to a minimum individual variations are great. I have seen tadpoles of the same age, from the same batch of eggs, reared in the same bowl, that varied as much as 20 mm. in length in five weeks' time. I quote this for what it may be worth, but it serves to indicate the complexity of the factors governing the growth of amphibian larvæ. I cannot but think that the results obtained by Professor Gudernatsch are attributable, in part at least, to some other factor besides thymus feeding.

In regard to the feeding experiments with thymus gland, either in the fresh state or powdered form, one point should not be overlooked, viz., that the growth-accelerating principles of the gland, if there be such, may undergo disintegration or suffer a change by contact with the digestive enzymes in the alimentary tract. Feeding experiments with thyroid glands show that no such changes occur, but possibly the same is not true of the thymus.

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